Inappropriate empirical antibiotic treatment results in poor outcomes in patients with severe sepsis and septic shock (Angus et al., 2001; Kumar et al., 2009). The administration of broad-spectrum antibiotics is therefore recommended in these critically ill patients, with a reassessment of antimicrobial therapy according to microbiological data for de-escalation when appropriate (Dellinger et al., 2013). The emergence of multidrug-resistant Enterobacteriaceae over the last 20 years represents a serious issue when it comes to the introduction of appropriate empirical antibiotic therapy. As β-lactams remain the drugs of choice in the treatment of Enterobacteriaceae, rapid detection of Enterobacteriaceae resistant to broad-spectrum cephalosporins, especially resistant to third-generation cephalosporins (3GCs), is becoming a major challenge in everyday practice.

Once blood cultures are positive, identification and antibiotic susceptibility testing (AST) of the causative pathogen by traditional methods take 24 and 48 h, respectively. Several methods have recently been described to speed up the identification and AST of Enterobacteriaceae. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS allows rapid identification directly either from the positive blood culture sample (Opota et al., 2015) or after 2–4 h subculturing on solid medium, with a 97.6 % success rate for the latter (Idelevich et al., 2014a; Verroken et al., 2015). Rapid methods, including the MALDI-TOF MS technology (Dortet et al., 2014) or chromogenic tests (Dortet et al., 2014a; Renvoise et al., 2013), MALDI-TOF MS (Microflex-Bruker Daltonics) and a β LACTA test were performed simultaneously on subcultures as described previously (Idelevich et al., 2014a; Renvoise et al., 2013). MALDI-TOF MS results were analysed using Biotyper 3.1.2 software (Bruker Daltonics) according to the manufacturer’s recommendations; however, we accepted scores as low as ≥1.7 for species identification performed on 3 h subcultures. Species identifications were confirmed by MALDI-TOF MS (scores ≥2) by resampling the same agar culture after 18–24 h incubation. β LACTA test results were compared with AST performed on bacterial colonies grown from blood cultures by using the disc-diffusion method on Mueller–Hinton agar (Bio-Rad). AST was interpreted according to the European Committee on Antimicrobial Susceptibility Testing clinical breakpoints (http://www.eucast.org/clinical_breakpoints/). MICs were determined using Etest (bioMérieux). ESBLs were detected using the double-disc synergy test (Jarlier et al., 1988). Cephalosporinase overexpression and acquired cephalosporinase were detected using Mueller–Hinton agar plates (bioMérieux) containing cloxacillin (250 mg l⁻¹). High-level penicillinases were characterized by high-level resistance (i.e. no inhibition diameter) to ticarcillin. Enterobacteriaceae were considered resistant to 3GCs when at least one 3GC (ceftazidime or cefotaxime) was resistant or intermediate according to the AST.

We prospectively collected blood cultures over a 4 month period at Hôpital Européen Georges Pompidou, an 827 bed teaching hospital in Paris, France. Blood culture broths were detected as positive using the BacT/ALERT system (bioMérieux). Two drops of each broth Gram stained as Gram-negative bacteria were then subcultured on Columbia agar supplemented with 5 % horse blood (bioMérieux). After 35 °C incubation for 3 h in air with 5 % CO₂, MALDI-TOF MS (Microflex-Bruker Daltonics) and a β LACTA test were performed simultaneously on subcultures as described previously (Idelevich et al., 2014a; Renvoise et al., 2013). MALDI-TOF MS results were determined using Etest (bioMérieux). Extended-spectrum β-lactamase (ESBL) producers were intermediate and/or resistant to third-generation cephalosporins from positive blood cultures using briefly incubated solid medium cultures.

Amongst the 109 Enterobacteriaceae isolated, 33 (30.3 %) were resistant to at least one 3GC and the resistance mechanisms are detailed in Table 1. ESBL producers were intermediate and/or resistant to both cefotaxime and
strains were resistant to ceftazidime and an acquired cephalosporinase. All five cases) and one (associated with impermeability in two
spp. overexpressing cephalosporinase associated with four
Enterobacteriaceae detected 28 of 33 3GC-resistant
108 subcultures after 3 h incubation and
The
b
harbouring a
Pseudomonas aeruginosa
b
mixed blood cultures had no impact on the
0.5 mg l
for which the MIC of cefotaxime was
ceftazidime, except for one
E. coli isolate
for which the MIC of cefotaxime was
0.5 mg l
−1.

The β LACTA test was performed on all
108 subcultures after 3 h incubation and
detected 28 of 33 3GC-resistant
Enterobacteriaceae (Table 1). The two
mixed blood cultures had no impact on the
β LACTA test results, including the one
harbouring a
Pseudomonas aeruginosa
isolette. The five false-negative results were
associated with four
Enterobacter
spp. overexpressing cephalosporinase (associated with impermeability in two
cases) and one
K. pneumoniae
harbouring an acquired cephalosporinase. All five
strains were resistant to ceftazidime and
cefotaxime, with MICs ranging from 8 to
>256 mg l
−1. For these five strains, the β
LACTA test was repeated with colonies
after 16–24 h incubation and still provided
negative results, thus showing that the
short incubation had no effect on
performances of the β LACTA test. No
false-positive results were observed as
all
Enterobacteriaceae
yielding a positive β
LACTA test were resistant to ceftazidime and/or cefotaxime, including one
Klebsiella
oxytoca
isolate overproducing its
chromosomal β-lactamase (MIC of
cefazidime: 2 mg l
−1).

Overall, for the detection of 3GC-resistant
Enterobacteriaceae, the β LACTA test had a
sensitivity of 84.8 %, a specificity of 100 %, a
positive predictive value of 100 % and
a negative predictive value of 94.0 %. These
results are close to those reported for
isolates grown for 16–24 h by Renvoise
et al. (2013), who demonstrated a
sensitivity and a specificity of 87.7 and
96.6 %, respectively. The failure to detect
an overexpressed cephalosporinase
phenotype is a well-known limit of the
β LACTA test (Defourny et al., 2014; Gallah
et al., 2014; Renvoise et al., 2013). As the
sensitivity for AmpC producers seems low,
a careful interpretation would be needed in
settings with a higher prevalence of
chromosomal AmpC overproducers or
pAmpC producers. When focusing on
ESBL-producing bacteria, the β LACTA
test had a sensitivity of 100 %, a specificity

<p>| Table 1. Results of MALDI-TOF MS and the β LACTA test on solid medium cultures incubated for 3 h compared with definitive identification and AST |
|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>MALDI-TOF MS identification</th>
<th>n</th>
<th>β LACTA test</th>
<th>MALDI-TOF MS identification</th>
<th>β-Lactam resistance phenotype</th>
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<tbody>
<tr>
<td>Proteus mirabilis</td>
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<td>E. coli</td>
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<tr>
<td></td>
<td>16</td>
<td>+</td>
<td>E. coli</td>
<td>ESBL*</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>–</td>
<td>E. coli</td>
<td>WT</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>E. coli†</td>
<td>WT + WT</td>
</tr>
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<td>–</td>
<td>Klebsiella pneumoniae</td>
<td>WT</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+</td>
<td>Klebsiella pneumoniae</td>
<td>ESBL*</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>Klebsiella pneumoniae</td>
<td>High-level penicillinase</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>Klebsiella pneumoniae + Enterobacter cloacae</td>
<td>WT + WT</td>
</tr>
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<td>Klebsiella oxytoca</td>
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<td>–</td>
<td>Klebsiella oxytoca</td>
<td>WT</td>
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<td>–</td>
<td>Citrobacter koseri</td>
<td>WT</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
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<td>–</td>
<td>Citrobacter freundii</td>
<td>High-level penicillinase</td>
</tr>
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<td>Cronobacter sakazakii</td>
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<td>–</td>
<td>Cronobacter sakazakii</td>
<td>WT</td>
</tr>
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<td>Enterobacter aerogenes</td>
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<td>WT</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>4</td>
<td>+</td>
<td>Enterobacter cloacae</td>
<td>ESBL*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>–</td>
<td>Enterobacter cloacae</td>
<td>WT</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>–</td>
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<td>Overexpressed cephalosporinase*</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>+</td>
<td>Enterobacter cloacae</td>
<td>Overexpressed cephalosporinase*</td>
</tr>
<tr>
<td>Morganella morganii</td>
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<td>+</td>
<td>Morganella morganii</td>
<td>Overexpressed cephalosporinase*</td>
</tr>
<tr>
<td>Pantoea agglomerans</td>
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<td>–</td>
<td>Pantoea agglomerans</td>
<td>High-level penicillinase</td>
</tr>
<tr>
<td>Serratia marcescens</td>
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<td>–</td>
<td>Serratia marcescens</td>
<td>WT</td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>+</td>
<td>E. coli</td>
<td>ESBL*</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>Klebsiella pneumoniae</td>
<td>WT</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>Klebsiella pneumoniae</td>
<td>Acquired cephalosporinase*</td>
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<tr>
<td>Not determined</td>
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<td>–</td>
<td>Enterobacter cloacae</td>
<td>WT</td>
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<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>Enterobacter cloacae</td>
<td>Overexpressed cephalosporinase*</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>E. coli</td>
<td>High-level penicillinase</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>E. coli</td>
<td>WT</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>+</td>
<td>Klebsiella pneumoniae</td>
<td>ESBL*</td>
</tr>
</tbody>
</table>

*Strains were resistant to at least one 3GC.†Blood culture broth also contained
Pseudomonas aeruginosa.
of 96.3 %, a positive predictive value of 90.3 % and a negative predictive value of 100 %.

Several rapid methods have been developed to accelerate the detection of 3GC-resistant Enterobacteriaceae, especially ESBL-producing Enterobacteriaceae. The β LACTA test has previously been successfully performed directly on positive blood cultures (Defourny et al., 2014). Similarly, the ESBL Nordmann/Dortet/Poirel test allows the detection of ESBL-producing Enterobacteriaceae directly from blood culture samples within 30 min (Dortet et al., 2015). However, both methods require several steps, including lysis of blood cells and centrifugation steps, which result in additional processing costs and are not easily integrated in the routine workflow. Thus, as previously illustrated (Lagacé-Wiens et al., 2012; Vlek et al., 2012; Wimmer et al., 2012), efficiency may require the preparation of batches of positive blood cultures before performing direct identification by MALDI-TOF MS: some laboratories could only perform it twice daily in everyday practice (Martiny et al., 2012), whilst others could not perform it during holidays (Vlek et al., 2012). Idelevich et al. (2014b) performed AST of positive blood cultures by inoculation of VITEK 2 cards with briefly incubated solid medium cultures with highly reliable results, but it required the VITEK 2 technology and the mean time to detection of 3GC-resistance was ~11 h.

In conclusion, performing the β LACTA test on 3 h incubated solid medium cultures from a positive blood culture is a rapid, reliable and inexpensive method to detect ESBL-producing Enterobacteriaceae. Neither supplementary steps of blood cell lysis or centrifugation nor additional consumables or special technical experience were required. Moreover, it can be easily integrated in the laboratory workflow as, at the same time and from the same plate, one sample was used to do the β LACTA test, whose result was obtained after 15 min incubation at room temperature, and another sample was used in parallel for MALDI-TOF MS identification. The impact of this rapid diagnostic test on therapeutic decisions and adjustments in empirical antibiotic therapy remains to be evaluated.

**References**


MS analysis after a 5-h subculture. 

